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Applicant UNIVERSITE LAVAL ET AL	

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Laurent	Bazinet	L'Ancienne-Lorette, Québec, Canada
David	Labbé	Sainte-Famille, Île d'Orléans, Québec, Canada
Angelo	Tremblay	Chartesbourg, Québec, Canada

☐ Additional inventors are being named on the 1 separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

METHOD FOR PURIFYING CATECHINS BY ELECTRODIALYSIS

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☒ Customer Number 020988 → 020988

OR

Type Customer Number here

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ENCLOSED APPLICATION PARTS (check all that apply)

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☐	Application Data Sheet, See 37 CFR 1.76		

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

		FILING FEE AMOUNT ($)
☐	Applicant claims small entity status. See 37 CFR 1.27	
☐	A check or money order is enclosed to cover the filing fee	
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Isabelle Chabot Date February 27, 2004

TYPED or PRINTED NAME ISABELLE CHABOT REGISTRATION NO. (if appropriate) 55,764

TELEPHONE (418) 640-5174 Docket Number: 6013-146USPR DAT/al

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14.

[SOR.PAT.FORM 110 – 02/2001]

OR File No. 6013-146USPR

METHOD FOR PURIFYING CATECHINS BY ELECTRODIALYSIS

TECHNICAL FIELD

[0001] The present invention relates to methods for purifying catechins by electrodialysis, and for reducing the electrical resistance on an uncharged membrane. The present invention also relates to the use of an uncharged membrane in a membrane arrangement for electrodialysis and to an electrodialysis apparatus comprising the same.

BACKGROUND OF THE INVENTION

[0002] Electrodialysisis (ED) is a membrane separation process in which ions species are induced to move by an electrical potential and are separated from water, macrosolutes and all uncharged solutes by means of ion-exchange membranes.

[0003] Ion-exchange membranes are traditionally highly distended gels containing polymers with a fixed ionic charge, allowing passage of anions or cations and very little else. To enlarge the use of ED purification to a wide range of molecules, ion-exchange membranes require optimization. It is however acknowledged that such optimisation involves majors tradeoffs between electrical resistance, selectivity and mechanical properties. This means that the membrane must be conductive to counterions and do not unduly restrict their passage through the membrane. For example, mechanical properties are improved with cross-link density, but so does the electrical resistance.

[0004] Since most of the ED membranes are made by chemical modification of polymers or by polymerization of functional monomers and cross linking agents, the pores formed by the interstices within the polymer have a random size and do not allow the purification of molecules based on their size. At the opposite, dialysis, ultrafiltration and nanofiltration membranes allows the purification of molecules based on size. They however appear useless in ED since their electrical resistance in an ED system would be inappropriately high. The purification of molecules based on their size and ED are therefore most of the time performed separately. U.S. Patents 4,043,896 and 4,123,342, Assignee Aqa-Chem, Inc. report an ultrafiltration and electrodialysis method and apparatus. These patents particularly report a

method and apparatus in which a solution to be treated is fed to one side of an ultrafiltration membrane cell, a concentration solution is delivered between a cation-selective and an ion-non-selective membrane, and an electric field is applied across the ED cell assembly. The ultrafiltration step sperates proteins while the application of an electric field increases demineralisation of the solution. The inventions described in these patents however have important drawbacks since they combine two distinct processes within the same apparatus. Indeed, the ultrafiltration process is generated by a pressure of 10 to 100 psi, exerted on the UF membrane, which therefore requires the membrane to be thicker in order to be resistant enough. Consequently, the overall resistance of the system is significantly increased. For example, such a system requires 6mm-thick ultrafiltration membranes that generate an overall resistance of 3.4 ohm-cm^2 in 1.0N Nacl. Therefore, this system is very demanding on energy consumption and is less interesting for industrial purposes.

[0005] As a consequence of the compromises between electrical resistance, selectivity and mechanical properties, ED has its greatest use in removing ions from solutions, such as salt from backish water and has not been used to purify molecules based on their size, even though it could theorically have been done with numerous charged molecules.

[0006] Catechins are colourless, water-soluble compounds that belong to flavonoids, a class of plant secondary metabolites widely distributed in the plant kingdom. Due to the presence of one or more electrical charges on the catechins molecule, ED would seem to have potential for extracting these compounds from green tea brewing. Indeed, the presence of hydroxyl groups on catechins and of ester groups on gallated catechins implies the possible presence of anionic charges at designated pH value. By circulating a green tea solution in an electrodialysis apparatus, it may be possible to use the driving force of the electrical field to force the catechins to migrate through the membrane out of the main body of the solution.

[0007] Since methods for purifying catechins reported in the prior art require the use of an organic solvent or adsorbent or are restricted to the purification of limited quantities of catechins, they are not easily transposable to the industrial scale since they are not environmentally nor economically advantageous.

[0008] Therefore, it would be desirable to be provided with a method for purifying catechins based on their size and electrical charge, that is economical and pollution free.

SUMMARY OF THE INVENTION

[0009] One aim of the present invention is to provide a method for purifying a catechin from a solution. The method according to the invention comprises submitting the solution to an electrical potential in a recipient, wherein the electrical potential induces the catechins to move through an ion exchange membrane arrangement. The membrane arrangement allows the selective enrichment of a second solution with the catechin. The present invention also provides the use of a uncharged membrane in a membrane arrangement for ED and a method for reducing the electrical resistance thereof. Finally, the present invention provides an electrodialysis apparatus that comprises an ion-exchange membrane arrangement, the latter comprising at least one uncharged membrane.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Further features and advantages of the present invention will become apparent from the following detailed description, taken in combination with the appended drawings, in which:

[0011] Figs. 1a and 1b show alternative configurations of electrodialysis membranes arrangements comprising anionic and UF membranes, respectively.

[0012] Fig. 2 is a curve of the pH of a green tea infusion as a function of electrodialysis time with different ion-exchange membranes.

[0013] Fig. 3 is a curve of the conductivity of a green tea infusion as a function of electrodialysis time with different ion-exchange membranes.

[0014] Fig. 4 is a curve of the electrical resistance of a green tea infusion as a function of electrodialysis time with different ion-exchange membranes.

[0015] Fig. 5 is a curve of the EGC concentration (µg/mL) of a green tea infusion as a function of electrodialysis time with different ion-exchange membranes.

[0016] Fig. 6 is a curve of the Caf concentration (µg/mL) of a green tea infusion as a function of electrodialysis time with different ion-exchange membranes.

[0017] Fig. 7 is a curve the EC concentration (µg/mL) of a green tea infusion as a function of electrodialysis time with different ion-exchange membranes.

[0018] Fig. 8 is a curve of the EGCG concentration (µg/mL) of a green tea infusion as a function of electrodialysis time with different ion-exchange membranes.

[0019] Fig. 9 is a curve of the GCG concentration (µg/mL) evolution of a green tea infusion as a function of electrodialysis time with different ion-exchange membranes.

[0020] Fig. 10 is a curve of the ECG concentration (µg/mL) of a green tea infusion as a function of electrodialysis time with different ion-exchange membranes.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0021] The present invention provides a method for purifying a catechin from a solution by electrodialysis. The method of the present invention is carried out by submitting the solution to an electrical potential in an electrodialysis cell, where the electrical potential induces the catechins to move through an ion exchange membrane arrangement. This membrane arrangement allows the enrichment of a second solution with a catechin of interest.

[0022] The method of the present invention may be used to purify any catechin from a solution but is preferably dedicated to purify EGC, EC, EGCG, GCG or ECG. A skilled artisan will understand that the method may also find uses in the purification of charged catechin-like molecules, such as caffeine.

[0023] The membrane arrangement may comprise homogenous-type ion exchange membranes or an heterogenous-type ion exchange membranes. The membrane may be a strongly acidic cation permeable membrane, a strongly basic anion permeable membrane (A), a strongly acidic cation permeable membrane (C) or a strongly basic anion selective membrane. These include, but are not limited to the following membranes: CMX, AMX, CL-25T, CM-1, CM-2, ACH-45T, AM-1, AM-2, AM-3, ACM, AMH, CMS, ACS, AFN, AFX, ACL E-5P, CLE-E, CGG-10F, CIMS,

CMH, C66-10F, ACS-3, CMB, AHA, CMV, CMO, AMV, ASV, ASO, AST, APS, DMV, CMT, CMS, AMT, ASS, AAV, AMP, AMD, DSV, AAV, HSV, CMD, HSF, A-101, A-171, A-201, A-211, K-101, K-171, K-172, MC 3470, MA 3475, MC 3142, MA 3148, 61AZ L386, 61AZ L389, 61CZ L386, 103QZ L386, 103PZ L386, 204SX ZL386, 204U3 86C-60, C-103C, C-313, A-60, A-104BR-4010, R-4035, R-1010, R-1035, CR 61 AZL 065, CR 61 AZL 183, CR 61 CZL 183, AR 103 PZL 065, AR 103 PZL 183, AR 103 QZL 219, AR 111 A, CRP, ARP, N117, N901, AQ CA-01, AQ CA-02, AQ AA-06, PC Acid 35, PC Acid 70, PC 100D, PC 200D, PC 400D, BP-1, AQ-BA-06, AQ-BA-04 or NEOSEPTA® AXE 01. However, as catechins and gallated catechins harbor anionic charges, due to the presence of hydroxyl or ester groups, it is an embodiment of the present invention to provide a membrane arrangement that comprises at least one anion-exchange membrane, such as PC400 D, AFN or AMX anion-exchange membrane.

[0024] It is also an embodiment of the present invention to provide a membrane arrangement that comprise at least one membrane having pores of a uniform size such as dialysis, ultrafiltration or nanofiltration membranes. Such membranes are preferably used since they can be selected according to the relative size of the molecule to be purified, contrarily to the majority of anionic or cationic membranes that comprise random pore sizes. It is an embodiment of the present invention to provide a membrane having pores of a uniform size that is uncharged. It is also an embodiment of the present invention to provide a conditioned uncharged membrane. A conditioned membrane may be obtained by immersing a non conditioned membrane in a salt solution, such as a sodium chloride solution or a potassium chloride solution, for at least five (5) minutes, and more preferably for at least one hour. The use of conditioned ultrafiltration membrane, for example, provides an advantage over non conditioned membranes since it contributes to significantly reduce the resistance of the membrane and thus, of the entire electrodialysis system.

[0025] The membrane arrangement of the present invention may comprise any arrangement of anions (A), cations (C), ions (I) and ultrafiltration (UF) membranes. For example, an electrodialysis cell may comprises a membrane arrangement such

as C/UF/I, C/UF/UF/C, C/UF/UF/UF/C, where the size of the pores of each UF is adapted so as to enhance the separation of the different molecules.

[0026] The first solution from which catechins may be purified may be a plant infusion and preferably a tea infusion and more preferably a green tea infusion. More particularly white tea leaves or green tea leaves are preferred since their EGCG content is the most interesting among plant products. The present invention is however not restricted to tea products. For example, fruits such as grape, apple, apricot , blackberry, or cherry or products derived from scutellaria and bamboo, could be used as raw material to obtain EGCG. The second solution in which catechins are found after being subjected to the electrodialysis process is preferably a salt solution, more preferably potassium chloride solution and even more preferably a 2 g/L KCl solution.

[0027] The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE 1

PURIFICATION OF CATECHINS FROM A GREEN TEA INFUSION

Materials and methods

Materials

[0028] The green tea was a non-biological Japanese green tea (lot 12423TKA) obtained from local retailer La Giroflée (Québec City, QC, Canada). The green tea was stored at room temperature in a dark and dry space. (-)-Epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechine gallate, (-)-gallocatechin gallate and caffeine standards were obtained from Sigma Company (Saint-Louis, MO, U.S.A.).

[0029] Three (3) anionic and one (1) ultrafiltration membranes, all commercially available, were selected according to their physico-chemical characteristics (see Table 1).

Table 1: Physico-chemical characteristics of the three anionic membranes and the ultrafiltration membrane.

	UF 1000 Da	AMX-SB	AFN	PC-400 D
Electrical resistance (Ω/cm^2)	N/D	2.0-3.5	0.2-1.0	10
Thickness (mm)		0.14-0.18	0.15-0.18	0.09-0.11
Burst strength (kg/cm^2)	N/D	4.5-5.5	2.0-4.0	4.0-5.0
Material	C	Polymer of polydivinylbenzene and polystyrene Minimal reinforcement		Nature non communicated Reinforced with polyester

Methods

Electrodialysis configuration

[0030] The module used was an MP type cell (100 cm2 of effective electrode surface) manufactured by ElectroCell (Täby, Sweden). The cell consisted of several compartments separated by cationic and tested membranes (Figure 1). The compartments defined three closed loops containing the solution to be treated (green tea brewing), an aqueous potassium chloride solution (5g/L KCl) and an electrolyte solution (20 g/L NaCl). Each closed loop was connected to a separate external reservoir to allow continuous recirculation of the solutions. The electrolytes were circulated using three centrifugal pumps, and the flow rates were controlled using flowmeters. The anode, a dimensionally-stable electrode (DSA), and the cathode, a 316 stainless-steel electrode, were supplied with the MP cell. The anode/cathode voltage difference was supplied by a variable 0-100 V power source.

Protocol

[0031] The capacity of the different membranes to enable the migration of catechins. was tested under the same conditions. Briefly, 20 g of green tea were brewed in 1000 mL of double-distilled water to solubilize catechins since it provides the 1:50 tea:water ratio suggested in the prior art . Tea leaves were brewed at 70°C for 40 minutes in a thermostated water-bath, quickly cooled down and stored at 4°C. until electrodialysis experiments.

[0032] Electrodialysis experiments were performed in a batch process with a constant current density of 1A, 6 liters of electrolyte and a 2.5 liter of green tea infusion, for 1 hour. The initial pH of the green tea infusions ranged from 5.6 to 5.8. Samples of green tea infusions were taken before applying electrical current to the apparatus, and every ten minutes during the electrodialysis process. Anode/cathode voltage difference, conductivity and temperature were recorded throughout the process. Concentrations of catechins and caffeine were determined by HPLC on samples stored at 4°C.

pH

[0033] The pH was mesured with a pH-meter model SP20 (epoxy gel combination pH electrode, VWR Symphony), from Thermo Orion (West Chester, PA, U.S.A.).

Conductivity

Conductivity was monitored at 4°C using a YSI conductivimeter (model 3100-115 V, Yellow Springs, OH) and an immersion probe (model 3417, k=1/cm, YSI).

HPLC method

[0034] The different samples of green tea infusion submitted to the electrodialysis process were filtered through a 0.20 µm filter (Aerodisc LC13 PVDF, Gelman Laboratory, Ann Arbor, MI) and diluted with HPLC grade water to be analyzed. Standard curves were calculated from a mix of flavanols and caffeine compounds at different concentrations: Correlations obtained ranged from 0.99808 to 0.99954. The RP-HPLC method was based on the National Institute of Standards and Technology method modified as follow :

Column : YMC-Pack ODS-AM, S-5 µm, 12 nm
Catalogue number : AM-303, AM12S05-2546WT
Dimensions and serial number : 250 x 4,6 mm I.D., N° 042568112(W)
Gel lot: 5531
Guard-column : YMC ODS-AM S-5 120Å 4,0 x 20mm DC Guard Cartridge
Particule number: AM12S050204WDA
Serial number : 502312331
Pump : Beckman, System Gold programmable solvent module 126
Detector : Beckman, System Gold programmable detector module 116

Auto-injector : LKB Bromma 2157 autosampler

Software : Gold v8.10

Phase A : Water + 0.05% TFA (purity > 99%, Laboratoire MAT, Québec, Canada)

Phase B : Acetonitrile (HPLC grade, EMD Chemicals inc., NJ) + 0.05% TFA (purity > 99%, Laboratoire MAT, Québec, Canada)

[0035] The detection of analytes was performed by UV detection at 210 nm. The column temperature was maintained at 40°C during analyses. Details on gradient used are listed in table 2. The mobile phases were filtered through a 0.2 µm nylon filter (Mendel Scientific Compagnie, Guelph, ON, Canada).

Table 2: *Gradient used for HPLC analysis*

Time (min)	%B
0	12.0
22	20.0
32	100.0
42	12.0

Statistical analysis

[0036] The experimental design is a complete randomized design with three repetitions. Data were subjected to an analysis of variance (ANOVA) using SAS sofware (Enterprise SAS Guide, Cary, NC, U.S.A.). Multiple comparisons tests (LSD) (lowest significant test) were performed to determine the significance of differences between membranes tested.

Results and discussion

pH

[0037] According to the variance analysis results, there was a significant effect of membrane type ($P<0.001$), duration ($P<0.001$) and dual interaction between membrane type and duration ($P<0.001$) on the pH throughout ED process (Fig. 2). The LSD tests showed that the differences observed with the different membrane types are significant ($P<0.001$). The pH varied similarly with the three anionic membranes, decreasing rapidly from an average value of 5.47 at the beginning to 2.92 after 30 minutes of treatment, to remain constant at an average value of 2.80 until the end of the ED process. Contrarily, the pH increased in a linear fashion from

5.42 at the beginning to pH 6.54 after 60 minutes of electrodialysis process with the UF-1000 Da membrane.

Conductivity

[0038] According to the ANOVA results, there was a significant effect of the membrane type ($P<0.001$) and duration ($P<0.001$) on conductivity evolution during ED treatment. There was also a significant dual-interaction between membranes type and duration ($P<0.001$) (Fig. 3).The LSD tests showed that ED treatments according to the type of membrane used were significantly different ($P<0.001$).

[0039] For the AFN and AMX-SB membranes, the evolution of the tea infusion conductivity was identical. It decreased rapidly from an averaged value of 742.00 µS/cm, at the beginning, to 463.50 µS/cm after 20 minutes of process. Then, tea conductivity slightly increased to 502.83 µS/cm after 30 minutes and remained constant at an averaged value of 536.20 µS/cm until the end of the ED process. The PC-400 Da membrane has a behaviour that is verysimilar to other anionic membranes but showed a lower average conductivity value for tea solution at the end of the process. Its conductivity decreased rapidly from an averaged value of 739.00 µS/cm, at the beginning of the electrodialysis, to 423.00 µS/cm after 20 minutes and then reached an average value of 448.25 µS/cm until the end of the treatment. At the opposite, tea conductivity increased in a linear fashion with the the UF-1000 Da membrane, from 701.33 µS/cm at the beginning to 766.33 µS/cm after 60 minutes of electrodialysis process.

System resistance

[0040] According to the ANOVA results, there is a significant effect of the treatments on the system resistance during the ED process ($P<0.001$). System resistance is influenced by the type of membrane used and the ED treatment duration because of the significant interaction between them ($P<0.001$) (Fig. 4). The LSD test show that there are significant differences between the ED treatments ($P<0.001$).

[0041] The evolution of the system resistance evolution was very similar for PC-400 Da and the AMX-SB. System resistance increased from an average value of 41.83 Ω when current was applied at the very beginning of the ED process to a

value of 58.83 Ω after 15 minutes, to remain constant until the end of the treatment. The system resistance increased from 28.33 Ω at the beginning, when current was applied, and then increased in a linear fashion to reach a value of 38.33 Ω after 60 minutes of treatment with AFN membrane. The UF-1000 Da behaviour was different from the anionic membranes. System resistance increased from 0 Ω at 0 minute to 53.67 Ω when current was applied and decreased in a linear fashion until a value of 47.00 Ω was reached at the end of the treatment.

[0042] A demineralization occured during the electrodialysis process with the three cationic/anionic configurations. This demineralization increased the overall system resistance. However, the AFN system resistance is lower than the two other membranes. This is due to the lower electrical resistance of the AFN membrane, 0.4-1.5 Ω•cm2 (Table 1). For the cationic/UF configuration, the migration of K+ cations to the tea compartment to keep the electroneutrality of the solution during the process likely contributes to slow down the decrease of the system resistance.

Catechins and caffeine

(-)-Epigallocatechin (EGC)

[0043] For the EGC, a membrane effect was observed (P<0.001) as a treatment duration effect (P<0.030) so a dual interaction of membrane and duration effect was observed (P<0.001). A second order equation was used to modelized the EGC migration behaviour (Fig. 5). There was a low concentration variation for the three anionic membranes, but the EGC concentration decreased by 50% with the UF-1000 Da membrane. Indeed, the EGC concentration decreased linearly from 1012.53 µg/mL at 0 min to 968.76 at 5 min, 943.95 µg/mL at 10 min, 878.06 µg/mL at 20 min, 787.31 µg/mL at 40 min and finally 518.27 µg/mL at 60 min. in the case of the UF-1000 Da membrane.

Caffeine (Caf)

[0044] A membrane effect was observed on caffeine (P<0.001) migration, but no duration effect has been detected (P>0.722). A linear equation was used to modelized the caffeine migration behaviour (Fig. 6). Results show there was no significant variation of caffeine concentration during the electrodialysis process. An average value, during the entire treatment, of 331.00 µg/mL with the AFN

membrane, 353.18 µg/mL with the AMX-SB membrane, 292.44 µg/mL with the UF-1000 Da membrane and 346.05 µg/mL with the PC-400 Da membrane was observed. Caffeine seems to remain in the tea compartment.

(-)-Epicatechin (EC)

[0045] A membrane effect was observed on EC migration ($P<0.001$) but not with duration ($P>0.723$). A linear equation was used to modelized the EC migration behavior (Fig. 7). No EC concentration drop has been observed with the AFN, AMX-SB and PC-400 Da membranes. Apparently, EC concentration decreased with the UF-1000 Da membrane toward 40 minutes, but this is not statistically signifiant.

(-)-Epigallocatechin gallate (EGCG)

[0046] A membrane effect ($P<0.001$), a slight duration effect ($P>0.066$) and dual effect ($P>0.079$) were observed on the EGCG migration. A second order equation was used to modelized the EGC migration behaviour (Fig. 8). A very low EGCG concentration variation for the electrodialysis treatments was observed with AFN, AMX-SB and PC-400 Da membranes while a very high EGCG concentration variation, around 50%, was observed with the UF-1000 Da membrane. So EGCG concentration with the UF-1000 Da membrane decreased from 891.92 µg/mL at 0 min to 825.82 µg/mL after 5 min, 861.57 µg/mL at 10 min, 865.91 µg/mL at 20 min, 679.75 µg/mL at 40 min and finally 445.70 µg/mL after 60 min of treatment. A migration delay was noted with EGCG. Indeed, it seems that EGCG electrodialysis effectively began after 20 minutes of process, the EGCG concentration staying relatively constant in the 20 first minutes and after the beginning of its linear migration.

(-)-Gallocatechin gallate (GCG)

[0047] The GCG electrodialysis showed a membrane ($P<0.001$) effect but no duration effect ($P>0.626$) was observed. A linear equation was used to modelized the caffeine migration behavior (Fig. 9). According to the GCG low concentration, very few variation were observed, whatever tmembrane type was used for the treatment. GCG variation during the treatment with the UF-1000 Da membrane was around 14%, but the appreciation of this decrease was difficult due to the high standard deviation values.

(-)-Epicatechin gallate (ECG)

[0048] For the ECG, a membrane effect ($P<0.001$) was observed but there was no noticeable duration effect ($P>0.209$). A second order equation was used to modelized the ECG migration behavior (Fig. 10). There was a very low ECG concentration variation during the treatment with the anionic membranes as with the UF-1000 Da membrane. The concentration variation between the treatment beginning and the end was not significant. Although there is no statistical significant difference for UF membrane, a slight decrease in ECG concentration after 30 minutes could be observed for a possible migration around 35%.

[0049] According to the results, the three anionic membranes do not show a statistical significant migration potential for the green tea catechins. AFN and AMX-SB membranes do not possess any pores that might allow the migration of organic molecules as big as catechins, which have a molecular weight of approximately 300,000 Da. No significant migration of catechins was observed with PC-400 Da membrane, even if it comprises pores that theoretically allow the passage of 400 Da molecules. This particular observation could be explain by the green tea acidification during the electrodialysis process that contribute to increase the catechins cationic charges and thus, to decrease the electrodialysis potential through such an anionic membrane.

[0050] For the UF-1000 Da membrane, an EGC and EGCG migration of 50% was observed after 60 minutes of treatment. In the case of GCG, a non statistically significant migration of approximately 14% was observed. ECG concentration seems to be decreased from 35% after 30 minutes of treatment and EC seems to be decreased after 40 minutes. This decrease is however not statistically significant. No caffeine migration was observed with the UF membrane. The green tea basification during the treatment increased the catechins anionic charges and thus, increased the migration potential through the UF-1000 Da membrane. Moreover, the appropriate size of the UF-1000 Da membrane apertures enables the catechins to freely migrate through it. This is the first demonstration that an electrodialysis method can be used as a method for purifying catechins.

[0051] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

OR File No. 6013-146USPR

WE CLAIM:

1. A method for purifying a catechin from a first solution that comprises mounting an ion exchange membrane arrangement in a recipient, placing said first solution in said recipient, submitting said first solution to an electrical potential in said recipient while said first solution is in said recipient, applying said electrical potential under conditions effective to induce said catechin to move through said ion exchange membrane arrangement in said recipient, said membrane arrangement having means allowing selective enrichment of a second solution with said catechin present therein at increased concentration.

2. The method of claim 1, wherein said membrane arrangement comprises at least one anion-exchange membrane.

3. The method of claim 2, wherein said at least one anion-exchange membrane is a PC400 D, AFN or AMX anion-exchange membrane.

4. The method of claim 1, wherein said membrane arrangement comprises at least one membrane having pores of a uniform size.

5. The method of claim 4, wherein said membrane having pores of a uniform size is a dialysis, an ultrafiltration or a nanofiltration membrane.

6. The method of claim 4, wherein said membrane having pores of a uniform size is an uncharged membrane.

7. The method of claim 6, wherein said uncharged membrane is conditioned.

8. The method of claim 7, wherein said conditioned membrane has been conditioned in a salt solution for at least five (5) minutes.

9. The method of claim 8, wherein said salt solution is a sodium chloride solution or a potassium chloride solution.

10. The method of claim 1, wherein said catechin is EGC, EC, EGCG, GCG or ECG.

11. The method of claim 1, wherein said first solution is a tea infusion.

12. The method of claim 11, wherein said tea infusion is a green tea infusion.

13. The method of claim 1, wherein said second solution is a salt solution.

14. The method of claim 13, wherein said salt solution is a potassium chloride solution.

15. Use of a membrane having pores of a uniform size in a ion-exchange membrane arrangement for electrodialysis.

16. The use of claim 15, wherein said membrane having pores of a uniform size is a dialysis, an ultrafiltration or a nanofiltration membrane.

17. The use of claim 15 , wherein said membrane having pores of a uniform size is an uncharged membrane.

18. The use of claim 17, wherein said uncharged membrane is conditioned.

19. The use of claim 18, wherein said conditioned membrane has been conditioned in a salt solution for at least five (5) minutes.

20. The use of claim 19, wherein said salt solution is a sodium chloride solution or a potassium chloride solution.

21. A method for reducing electrical resistance of an uncharged membrane that comprises conditioning said uncharged membrane in a salt solution for at least five (5) minutes.

22. The method of claim 21, wherein said salt solution is a sodium chloride solution or a potassium chloride solution.

23. An electrodialysis apparatus that comprises a container for a liquid, and an ion-exchange membrane arrangement mounted therein, wherein said membrane arrangement comprises at least one membrane having pores of a uniform size.

24. The electrodialysis apparatus of claim 23, wherein said membrane having pores of a uniform size is a dialysis, an ultrafiltration or a nanofiltration membrane.

25. The electrodialysis apparatus of claim 23, wherein said membrane having pores of a uniform size is an uncharged membrane.

26. The electrodialysis apparatus of claim 25, wherein said uncharged membrane is conditioned.

27. The electrodialysis apparatus of claim 26, wherein said conditioned membrane have been conditioned in a salt solution for at least five (5) minutes.

28. The electrodialysis apparatus of claim 27, wherein said salt solution is a sodium chloride solution or a potassium chloride solution.

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ABSTRACT

The present invention provides a method for purifying a catechin from a solution by electrodialysis, using a membrane arrangement the comprise at least one anion exchange membrane or uncharged membrane. The present invention also provides the use of a uncharged membrane and a method for reducing the electrical resistance thereof. Finally, the present invention provides an electrodialysis apparatus that comprises an ion-exchange membrane arrangement, the latter comprising at least one uncharged membrane.

Fig. 1

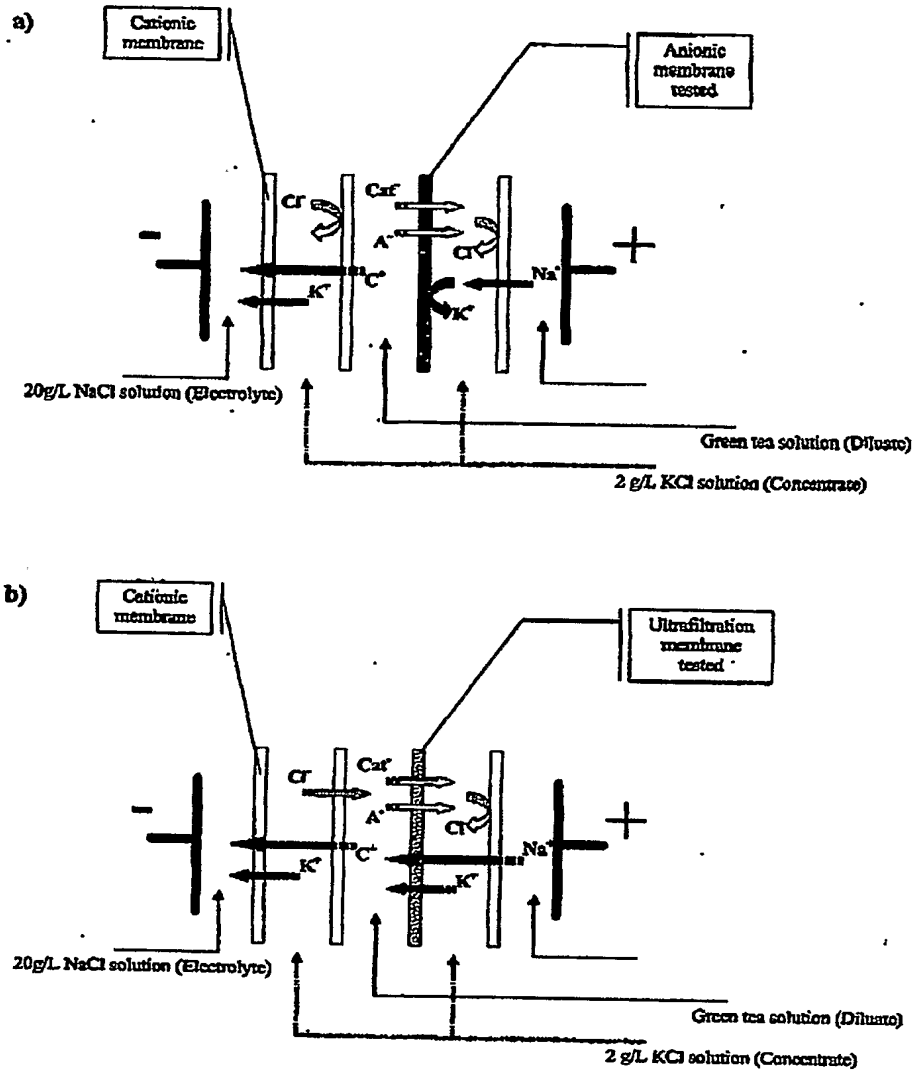

Fig. 2

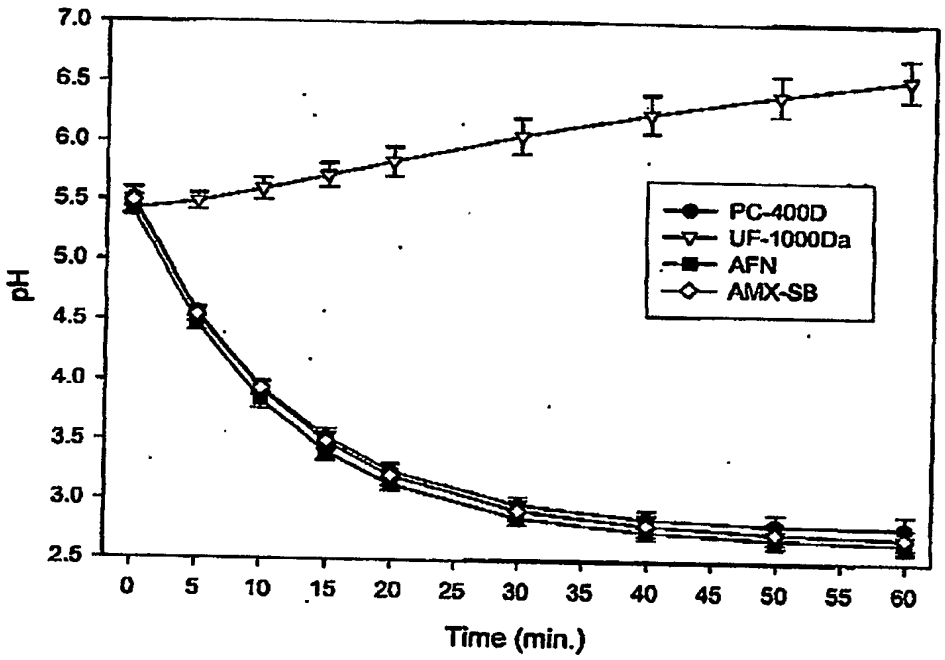

Fig. 3

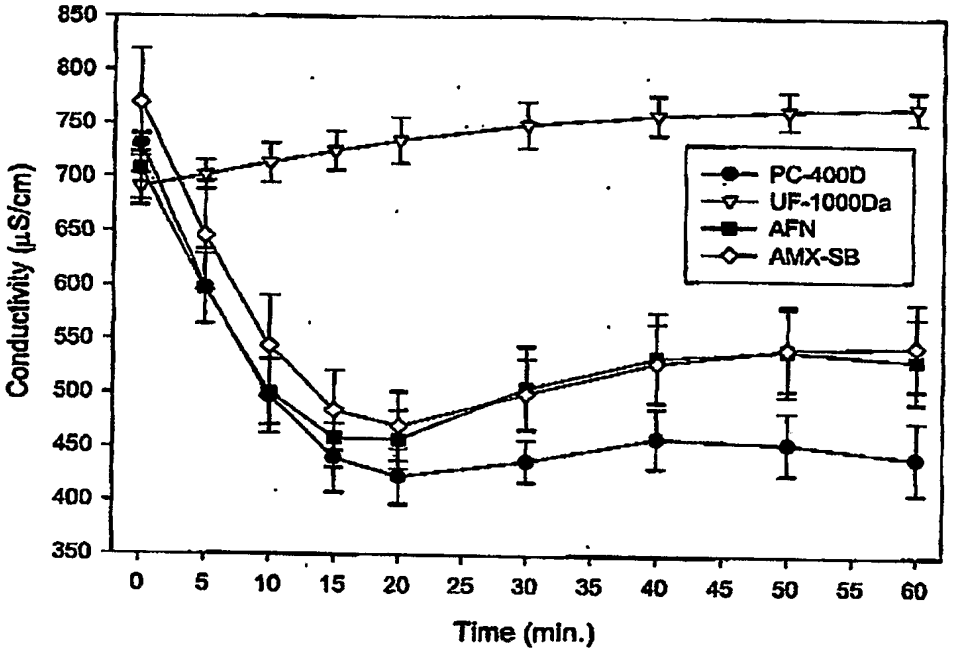

Fig. 4

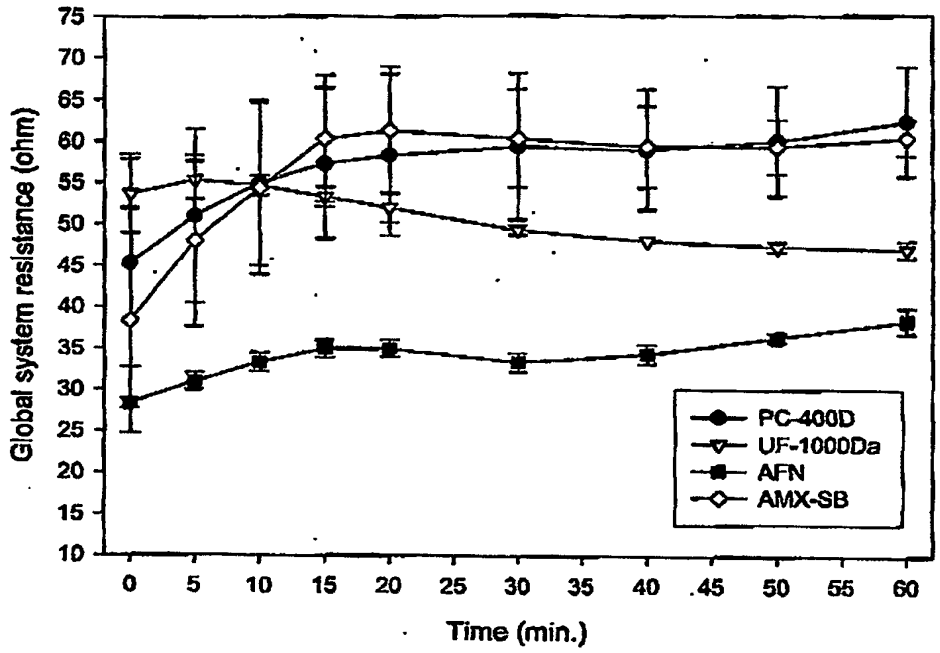

Fig. 5

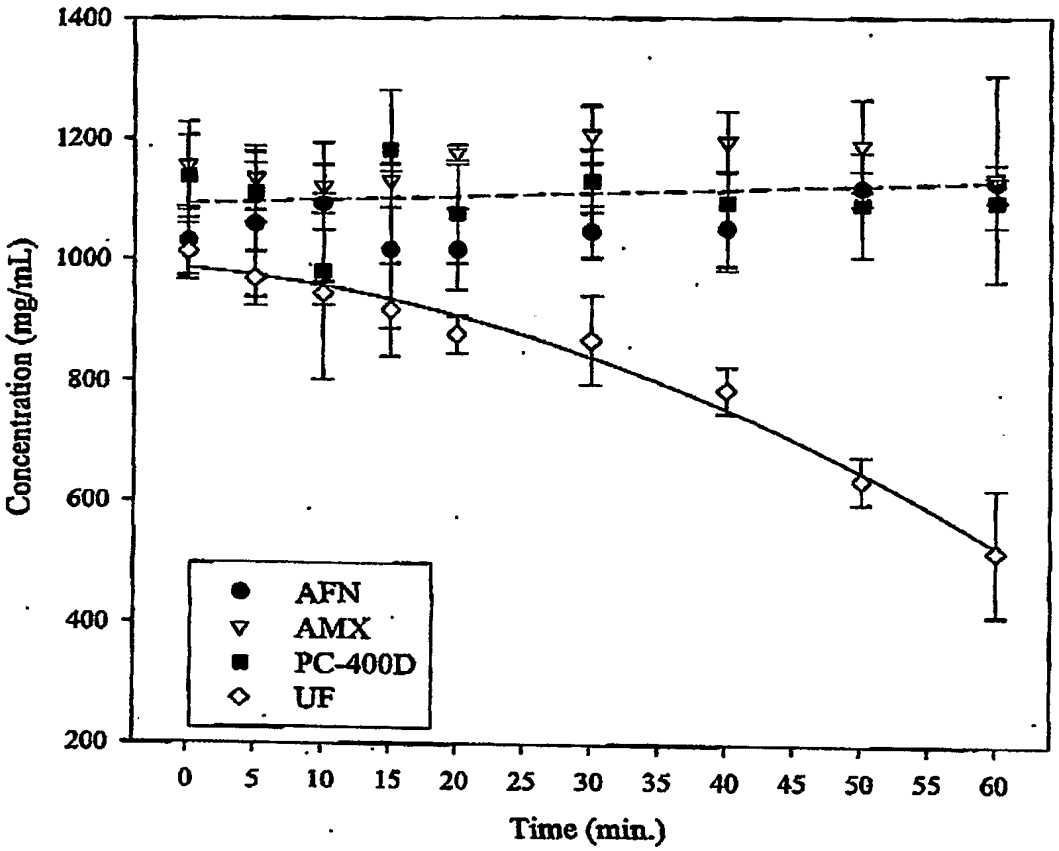

Fig. 6

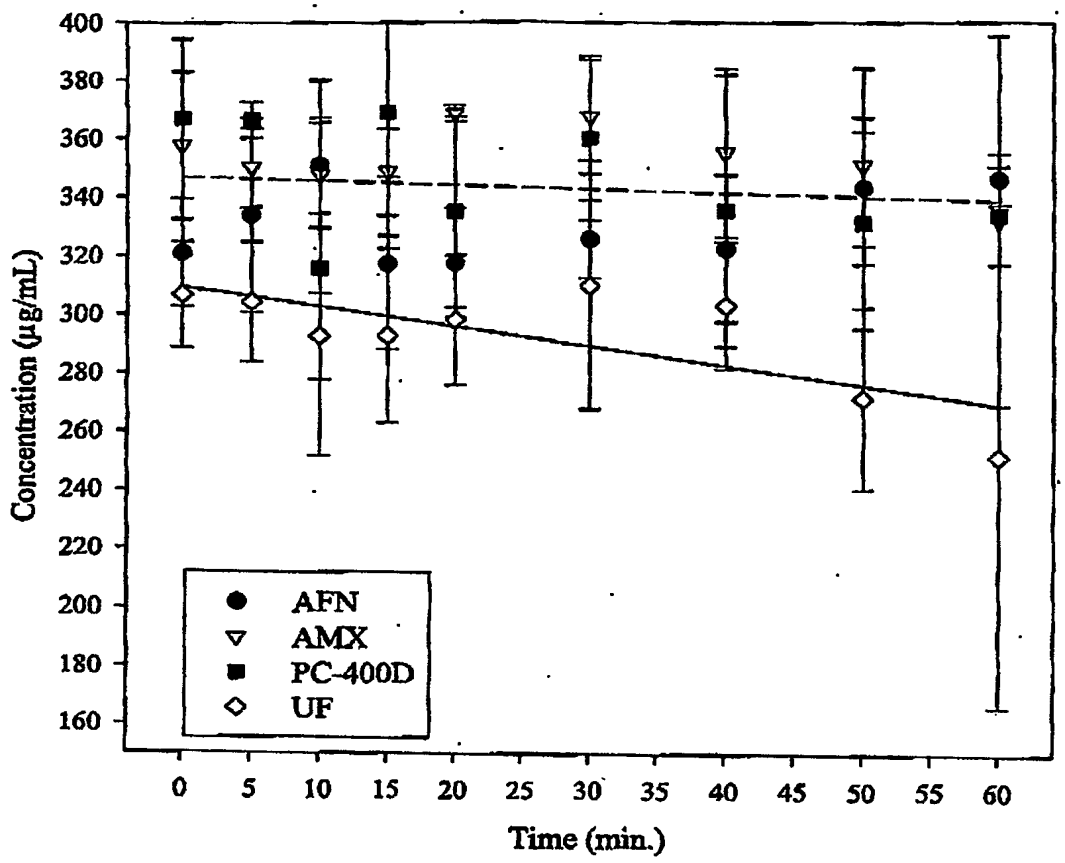

Fig. 7

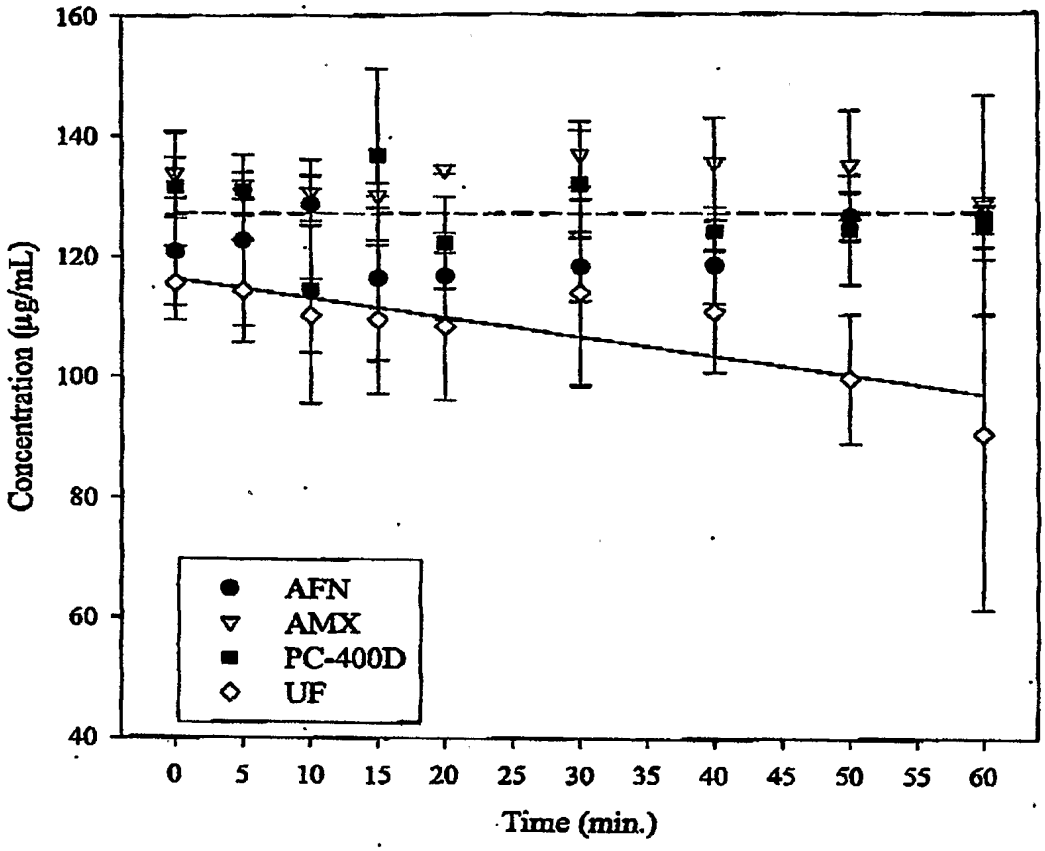

Fig. 8

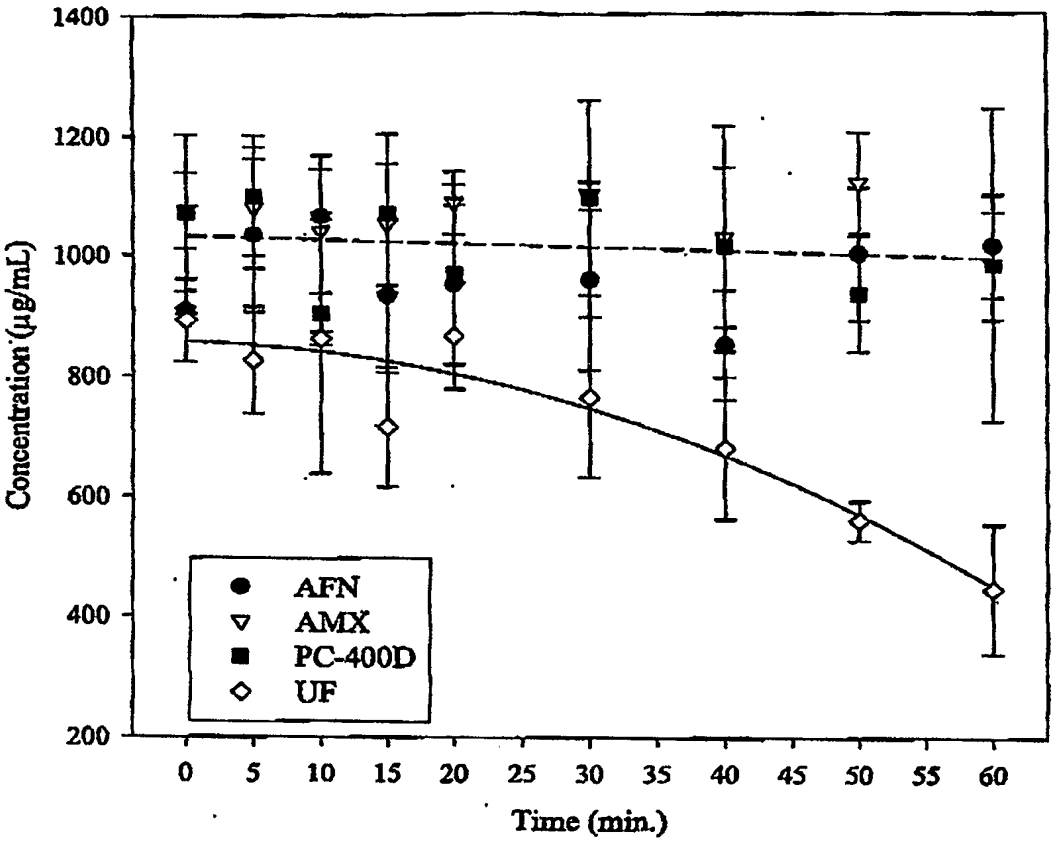

Fig. 9

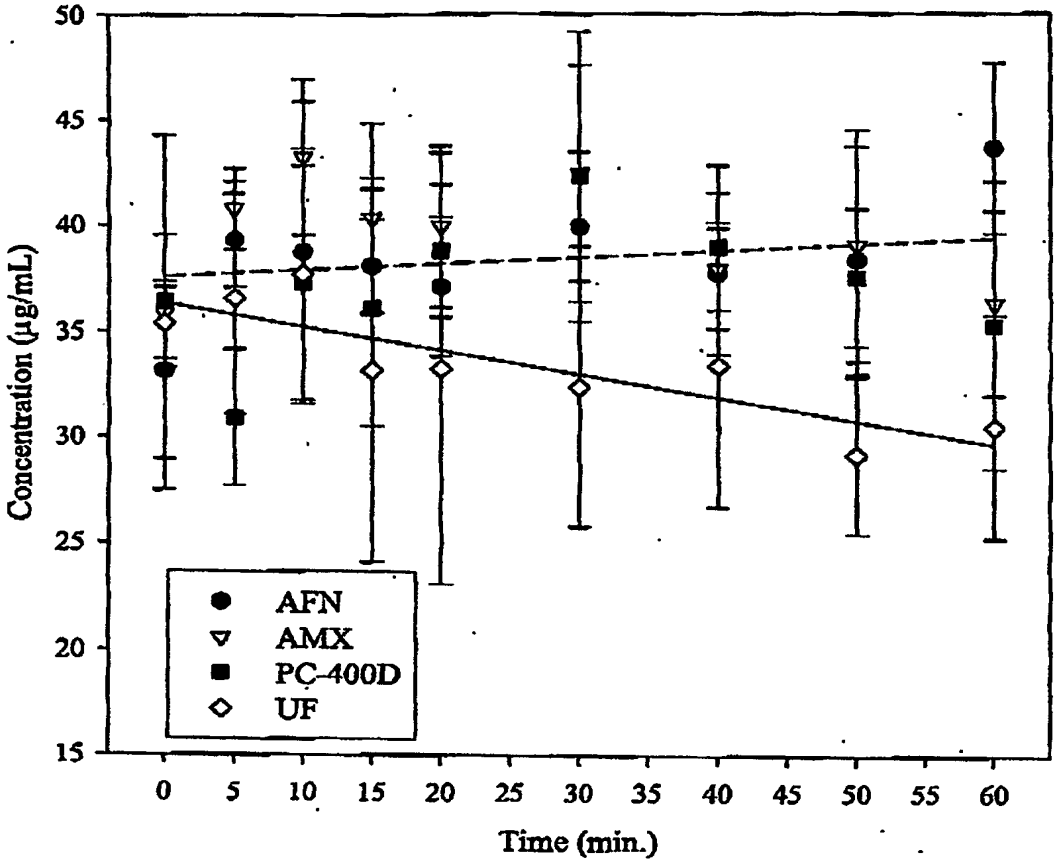

Fig. 10